nature neuroscience

A tarantula peptide against pain via ASIC1a channels and opioid mechanisms

Michel Mazzuca[1,4], Catherine Heurteaux[1,4], Abdelkrim Alloui[2,4], Sylvie Diochot[1], Anne Baron[1], Nicolas Voilley[1], Nicolas Blondeau[1], Pierre Escoubas[3], Agnès Gélot[2], Anny Cupo[1], Andreas Zimmer[3], Anne M Zimmer[3], Alain Eschalier[2] & Michel Lazdunski[1]

Psalmotoxin 1, a peptide extracted from the South American tarantula *Psalmopoeus cambridgei*, has very potent analgesic properties against thermal, mechanical, chemical, inflammatory and neuropathic pain in rodents. It exerts its action by blocking acid-sensing ion channel 1a, and this blockade results in an activation of the endogenous enkephalin pathway. The analgesic properties of the peptide are suppressed by antagonists of the μ and δ-opioid receptors and are lost in *Penk1*$^{-/-}$ mice.

Improving the treatment and the prevention of acute and chronic pain is an important goal for modern medicine. Ion channels have recently become very attractive targets in the search of new analgesics[1]. Acid-sensing ion channels (ASICs) are expressed throughout the central and peripheral nervous systems[1–5]. ASIC1a, ASIC1b, ASIC2b and ASIC3 are abundantly expressed in small DRG neurons, where ASIC3 is predominently implicated in a variety of pain sensations[6–8]. ASIC1a, ASIC2a and ASIC2b are abundant in brain and spinal cord neurons[2–8]. The most potent pharmacological agent for ASIC channels is psalmotoxin 1 (PcTx1). This peptide, which was isolated from a tarantula venom[9], potently and specifically inhibits homomeric ASIC1a channels. PcTx1 is not lethal, even at high concentrations, and could be a candidate for therapeutic applications. PcTx1 blocks ASIC1a channels in brain neurons[9], as well as in nociceptors[10]. It also blocks ASIC1a channels in spinal neurons (see **Supplementary Results** and **Supplementary Fig. 1** online), which permits the use of intrathecal injections.

We first evaluated the effects of PcTx1 on behavioral reactions in acute pain models (see Supplementary Methods online). In tail immersion and hot plate tests, a large antinociceptive effect was observed following both intrathecal and intracerebroventricular (ICV) injections of PcTx1 (**Fig. 1**). Intraperitoneal or subcutaneous injections had no effect, which was expected, as PcTx1 is a peptide. Morphine induced an antinociceptive effect very similar to that of PcTx1 (**Fig. 1a,b,d**). A similar antinociceptive effect was also produced by intrathecal injection of specific antisense nucleotides (**Fig. 1c**) that have previously been used successfully[11] to eliminate ASIC1a expression in the retina. We used a formalin test to investigate the PcTx1 effects (intrathecal) in

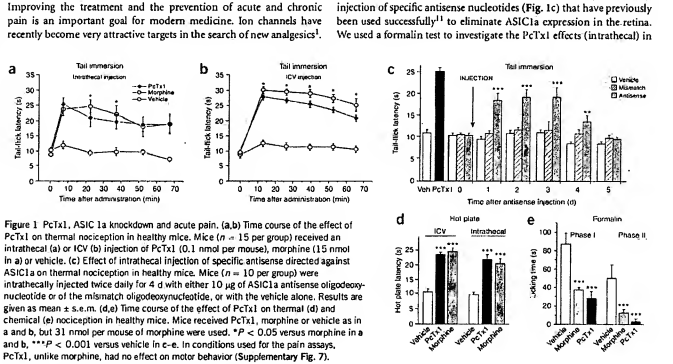

Figure 1 PcTx1, ASIC 1a knockdown and acute pain. (**a**,**b**) Time course of the effect of PcTx1 on thermal nociception in healthy mice. Mice ($n = 15$ per group) received an intrathecal (a) or ICV (b) injection of PcTx1 (0.1 nmol per mouse), morphine (15 nmol in a) or vehicle. (**c**) Effect of intrathecal injection of specific antisense directed against ASIC1a on thermal nociception in healthy mice. Mice ($n = 10$ per group) were intrathecally injected twice daily for 4 d with either 10 μg of ASIC1a antisense oligodeoxynucleotide or of the mismatch oligodeoxynucleotide, or with the vehicle alone. Results are given as mean ± s.e.m. (**d**,**e**) Time course of the effect of PcTx1 on thermal (d) and chemical (e) nociception in healthy mice. Mice received PcTx1, morphine or vehicle as in a and b, but 31 nmol per mouse of morphine were used. $^*P < 0.05$ versus morphine in a and b, $^{***}P < 0.001$ versus vehicle in c–e. In conditions used for the pain assays, PcTx1, unlike morphine, had no effect on motor behavior (**Supplementary Fig. 7**).

[1]Institut de Pharmacologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique (CNRS), Institut Paul Hamel, Université de Nice-Sophia Antipolis, 660 Route des Lucioles 06560 Valbonne, France. [2]Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 766, Faculté de Médecine, CHU, Clermont-Ferrand, France. [3]University of Bonn, Department of Molecular Psychiatry, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany. [4]These authors contributed equally to this work. Correspondence should be addressed to M.L. (lpmic@ipmc.cnrs.fr).

Received 4 June; accepted 22 June; published online 15 July 2007; doi:10.1038/nn1940

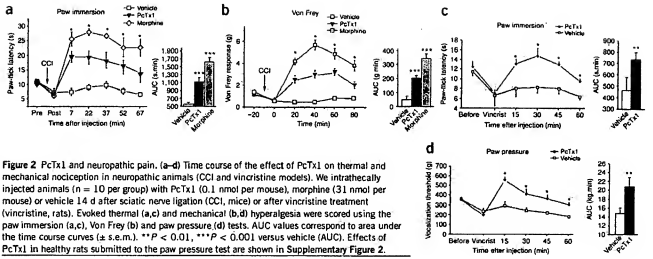

Figure 2 PcTx1 and neuropathic pain. (a–d) Time course of the effect of PcTx1 on thermal and mechanical nociception in neuropathic animals (CCI and vincristine models). We intrathecally injected animals ($n = 10$ per group) with PcTx1 (0.1 nmol per mouse), morphine (31 nmol per mouse) or vehicle 14 d after sciatic nerve ligation (CCI, mice) or after vincristine treatment (vincristine, rats). Evoked thermal (a,c) and mechanical (b,d) hyperalgesia were scored using the paw immersion (a,c), Von Frey (b) and paw pressure (d) tests. AUC values correspond to area under the time course curves (± s.e.m.). $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle (AUC). Effects of PcTx1 in healthy rats submitted to the paw pressure test are shown in Supplementary Figure 2.

irritant chemical nociception (acute, phase I) and in inflammation (late, phase II) (**Fig. 1e**). In phase I (0 to 5 min), morphine and PcTx1 had comparable antinociceptive effects. In phase II (from 30 to 40 min), the score reduction induced by PcTx1 was even higher than that induced by morphine ($P < 0.05$). These three acute pain models are indicative of a potent antinociceptive effect of PcTx1.

We then analyzed the effects of PcTx1 in two persistent pain models (see **Supplementary Methods**). Mononeuropathy caused by a chronic constriction injury (CCI) of sciatic nerve in rat produced a hyperalgesic static and tactile allodynia (**Fig. 2a,b**). Similar to morphine, PcTx1 reversed both the thermal hyperalgesia and the tactile allodynia. On healthy rats, PcTx1 only slightly increased the mechanical threshold (**Supplementary Fig. 2** online). We also assessed PcTx1 action using a mouse vincristine-induced neuropathy model (see **Supplementary Methods**). Vincristine is an anticancer drug that is unfortunately associated with peripheral neuropathy. Vincristine induced hypersensitivity to heat (**Fig. 2c**) and to mechanical stimuli (**Fig. 2d**), which was completely abolished by PcTx1 (**Fig. 2c,d**).

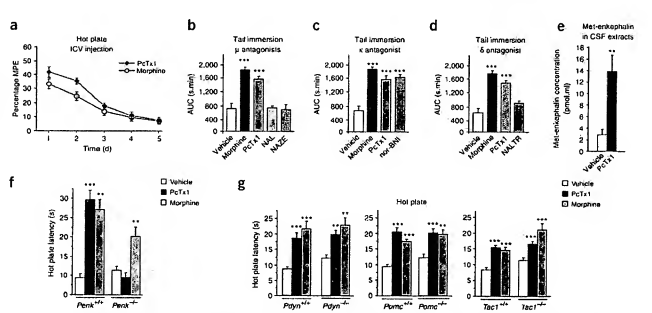

Figure 3 Opioid receptors and enkephalins are involved in the antinociceptive effect of PcTx1. (a) Repeated ICV injections of PcTx1 or morphine into healthy mice results in tolerance ($n = 10$ per group). MPE, maximum possible effect. (b–d) Effects of pretreatment with μ (b), κ (c) and δ opioid (d) antagonists on tail immersion responses produced by ICV administration of PcTx1 (0.1 nmol, $n = 12$ per group). NAL, naloxone; NAZE, naloxonazine; NALTR, naltrindole; injected before PcTx1. Morphine (15 nmol per mouse, ICV). PcTx1 (0.1 nmol per mouse; ICV) and vehicle (ICV) were injected alone as controls. $^{***}P < 0.001$ versus vehicle. (e) Effect of PcTx1 (0.1 nmol per mouse, ICV) on met-enkephalin concentration (pmol per ml) in cerebrospinal fluid (CSF). (f,g) Comparison of nociceptive behavior of wild-type, *Penk1*$^{-/-}$ (f), *Pdyn*$^{-/-}$, *Pomc*$^{-/-}$ and substance P (*Tac1*$^{-/-}$) (g) mice to noxious thermal stimulus (52 °C). Mice ($n = 10$ per group) received an intrathecal injection of PcTx1 (0.1 nmol per mouse), morphine (31 nmol per mouse) or vehicle. Results are given as mean ± s.e.m. $^{***}P < 0.001$, $^{**}P < 0.01$ versus vehicle. NAL, NAZE, nor-BNI and NALTR were without effect by themselves (**Supplementary Fig. 4**).

Because there is no additivity between the analgesic effects of morphine and PcTx1 (**Supplementary Fig. 3** online) and because PcTx1 treatment, similar to morphine treatment, results in tolerance (**Fig. 3a**), we asked whether the analgesic ICV effect of PcTx1 was inhibited by naloxone, a nonspecific antagonist of the opioid receptors, by naloxonazine, a specific antagonist of μ1-opioid receptors, by naltrindole, a δ-receptor antagonist, and by nor-binaltorphimine (nor-BNI), a κ-opioid receptor antagonist, both without any effect by themselves (**Supplementary Fig. 4** online). The inhibitory effects of naloxone, naloxonazine and naltrindole indicated that PcTx1 produces its antinociceptive effect via the stimulation of μ- and δ-opioid receptors (**Fig. 3b–d**). ASIC1a was coexpressed with Met-enkephalin in the dorsal horn neurons (**Supplementary Fig. 5** online). Enkephalins, endogenous opioid peptides, are potent ligands of both μ and δ receptors[12], and are involved in the analgesic effect of PcTx1. ICV injection of PcTx1 (like injection of ASIC1a antisense nucleotides, **Supplementary Fig. 6** online) increased Met-enkephalin levels in the cerebrospinal fluid (**Fig. 3e**), and the analgesic effect of PcTx1 was lost in mice deficient for the *preproenkephalin* gene ($Penk1^{-/-}$ mice) (**Fig. 3f**), but not in mice lacking either the *pro-opiomelanocortin-α* ($Pomc^{-/-}$) or *prodynorphin* ($Pdyn^{-/-}$) genes, or in mice deficient for *tachykinin* ($Tac1^{-/-}$) (**Fig. 3g**).

These results suggest that the ASIC1a channel is an important molecular target for treating both acute and neuropathic pain and that PcTx1 itself could be a new specific analgesic drug working upstream of the opiate receptors. Being a peptide, PcTx1 would have to be injected intrathecally to patients, similarly to ω-conotoxin MVIIA, a peptide originating from cone snail venom, which blocks high-voltage activated Ca^{2+} channels and has been approved for use in human patients[13]. Knowing that PcTx1 has analgesic effect and that it does not show some of the secondary effects of morphine (**Supplementary Fig. 7** online) will also encourage the search for nonpeptidic blockers of the ASIC1a channel, which can be taken orally. Our work also highlights the connection between ASIC1a and the endogenous opioid system (**Fig. 3**). ASIC1a blockade leads to the activation of the enkephalin system, but the mechanistic details by which this happens are not yet known. Notably, ASIC1a is also modulated by peptides of the FMRF family[5,8,14], which are abundant in brain and spinal cord and have anti opioid properties[15], and is inhibited by PcTx1, which displays opioid-like properties against pain. It is thus tempting to correlate these observations and to suggest that ASIC1 channels are at a crossroad between the enkephalin and the FMRF-related peptides pathways. It is also tempting to suggest that FMRF-like peptides negatively control the antinociceptive opiate pathway through the ASIC1a channel. This negative control would then disappear when ASIC1a is blocked by PcTx1. Hopefully, this may open a new avenue of research into the endogenous opioid mechanisms in nociception.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We are very grateful to M. Jodar and Y. Benhamou for expert technical assistance and to A. Patel for the reading of the manuscript. This work was supported by the CNRS, the Agence Nationale de la Recherche (Projet Analgésie et canaux Ioniques dans les voies Nociceptives), the Paul Hamel Institute and the Association Française contre les Myopathies.

AUTHOR CONTRIBUTIONS

M.M. and C.H. did the initial observations and conducted a large part of the experiments, analyzed the data and participated to the preparation of the manuscript. A.A., S.D., A.G. and A.E. conducted pain experiments both with PcTx1 and the antisense strategy. A.B. conducted localization and electrophysiological experiments and helped in the preparation of the manuscript. P.E. produced and provided PcTx1. N.V. was responsible for the localization and colocalization experiments. N.B. was associated with experiments using opiate antagonists. A.C. measured met-enkephalin concentrations in the cerebrospinal fluid. A.Z. and A.M.Z. provided the $Enk^{-/-}$, $Dyn^{-/-}$ and $Tac1^{-/-}$ mice. M.L. supervised the project and wrote the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

Published online at http://www.nature.com/natureneuroscience
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Wood, J.N. *Handb. Exp. Pharmacol.* **177**, 329–358 (2007).
2. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. & Lazdunski, M. *Nature* **386**, 173–177 (1997).
3. Waldmann, R. & Lazdunski, M. *Curr. Opin. Neurobiol.* **8**, 418–424 (1998).
4. Krishtal, O. *Trends Neurosci.* **26**, 477–483 (2003).
5. Wemmie, J.A., Price, M.P. & Welsh, M.J. *Trends Neurosci.* **29**, 578–586 (2006).
6. Sutherland, S.P., Benson, C.J., Adelman, J.P. & McCleskey, E.W. *Proc. Natl. Acad. Sci. USA* **98**, 711–716 (2001).
7. Sluka, K.A. *et al. Pain* **129**, 102–112 (2007).
8. Lingueglia, E., Deval, E. & Lazdunski, M. *Peptides* **27**, 1138–1152 (2006).
9. Escoubas, P. *et al. J. Biol. Chem.* **275**, 25116–25121 (2000).
10. Mamet, J., Baron, A., Lazdunski, M. & Voilley, N. *J. Neurosci.* **22**, 10662–10670 (2002).
11. Ettaiche, M., Deval, E., Cougnon, M., Lazdunski, M. & Voilley, N. *J. Neurosci.* **26**, 5800–5809 (2006).
12. Kieffer, B.L. & Gaveriaux-Ruff, C. *Prog. Neurobiol.* **66**, 285–306 (2002).
13. Lewis, R.J. & Garcia, M.L. *Nat. Rev. Drug Discov.* **2**, 790–802 (2003).
14. Wemmie, J.A. *et al. Neuron* **34**, 463–477 (2002).
15. Roumy, M. & Zajac, J. *Eur. J. Pharmacol.* **345**, 1–11 (1998).